Genetic basis of channelopathies and cardiomyopathies in Hong Kong Chinese patients: a 10-year regional laboratory experience

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ABSTRACT

Introduction: Hereditary channelopathies and cardiomyopathies are potentially lethal and are clinically and genetically heterogeneous, involving at least 90 genes. Genetic testing can provide an accurate diagnosis, guide treatment, and enable cascade screening. The genetic basis among the Hong Kong Chinese population is largely unknown. We aimed to report on 28 unrelated patients with positive genetic findings detected from January 2006 to December 2015.

Methods: Sanger sequencing was performed for 28 unrelated patients with a clinical diagnosis of channelopathies or cardiomyopathies, testing for the following genes: *KCNQ1, KCNH2, KCNE1, KCNE2,* and *SCN5A*, for long QT syndrome; *SCN5A* for Brugada syndrome; *RYR2* for catecholaminergic polymorphic ventricular tachycardia; *MYH7* and *MYBPC3* for hypertrophic cardiomyopathy; *LMNA* for dilated cardiomyopathy; and *PKP2* and *DSP* for arrhythmogenic right ventricular dysplasia/cardiomyopathy.

Results: The study included 17 male and 11 female patients; their mean age at diagnosis was 39 years (range, 1-80 years). The major clinical presentations included syncope, palpitations, and abnormal electrocardiography findings. A family history was present in 13 (46%) patients. There were 26 different heterozygous mutations detected, of which six were novel—two in *SCN5A* (NM_198056.2:c.429del and c.2024-11T>A), two in *MYBPC3* (NM_000256.3:c.906-22G>A and c.2105_2106del), and two in *LMNA* (NM_170707.3:c.73C>A and c.1209_1213dup).

Conclusions: We characterised the genetic heterogeneity in channelopathies and cardiomyopathies among Hong Kong Chinese patients in a 10-year case series. Correct interpretation of genetic findings is difficult and requires expertise and experience. Caution regarding issues of non-penetrance, variable expressivity, phenotype-genotype correlation, susceptibility risk, and digenic inheritance is necessary for genetic counselling and cascade screening.

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New knowledge added by this study
- We characterised the genetic heterogeneity in channelopathies and cardiomyopathies among Hong Kong Chinese patients and described 26 mutations with six novel variants.
- This is the first case series of cardiac genetics in Hong Kong.

Implications for clinical practice or policy
- This study provides genetic information for variant interpretation and insight into the clinical application of genetic testing for channelopathies and cardiomyopathies.

Introduction

Cardiac genetics is evolving rapidly and many new insights have recently been achieved. Genetic causes are found in various potentially lethal channelopathies and cardiomyopathies including long and short QT syndrome (LQTS and SQTS), Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C), Barth syndrome, and left ventricular non-compaction.[1] Knowledge of genetics deepens the understanding of pathophysiology and remarkably changes the diagnosis, treatment, and genetic counselling for recurrence risk and family planning. This group is highly genetically heterogeneous (Table 1[2]).

The genetic basis of inherited cardiac diseases in the Hong Kong Chinese population is largely unknown. The Princess Margaret Hospital provides a comprehensive cardiac genetic service. We conducted this study to review the clinical and genetic findings of 28 unrelated positive cases encountered between January 2006 and December 2015.

Methods

Diagnosis of the cardiac conditions was based on clinical assessments by a cardiologist and practice guidelines.[3-5] The patients were referred by cardiologists from various public hospitals for genetic analysis. Only patients with positive genetic findings are reported in this study. There were seven patients with LQTS, two with Brugada syndrome, two with CPVT, nine with HCM, four with DCM, and four with ARVD/C. Local ethics board approval was obtained. Peripheral blood samples were collected from the proband after informed consent was obtained. Genomic DNA was extracted using

a QIAamp Blood Kit (Qiagen, Hilden, Germany). The coding exons and the flanking introns (10 bp) of each gene were amplified by polymerase chain reaction. The primer sequences and protocol are available on request. Sanger sequencing was

香港華籍患者的心臟離子通道病和心肌病基因檢測的情況：一所分區實驗室的經驗

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引言：遺傳性心臟離子通道病和心肌病可以致命，它們的臨床表現多樣化，具遺傳異質性，並至少與90個基因有關。基因檢測可以提供準確診斷，指導治療，並進行直系家屬的連帶基因篩檢。香港華籍人口在這兩種病的基因變異分佈尚未清楚。本文描述2006年1月至2015年12月期間，接受基因檢查並呈陽性結果的28名患者；他們並沒有直接的血緣關係。

方法：通過Sanger測序，替沒有血緣關係的28名心臟離子通道病或心肌病患者進行以下基因檢查：長QT綜合徵的KCNQ1、KCNH2、KCNE1、KCNE2和SCN5A基因；Brugada綜合症的SCN5A基因；兒茶酚胺敏感性多形性室速的RYR2基因；肥厚型心肌病的MYH7和MYBPC3基因；擴張型心肌病的LMNA基因；致心律失常性右室心肌病的PKP2和DSP基因。

結果：患者17男和11女的平均確診年齡為39歲（介乎1-80歲）。主要臨床表現包括暈厥、心悸和心電圖異常。13名患者（46.4%）有家族史。基因檢測結果發現有26個不同的基因變異，其中有6個屬新發現：有兩個在SCN5A基因中（NM_198056.2：c.429del和c.2024-11T>A），兩個在MYBPC3基因中（NM_000256.3：c.906-22G>A和c.2105_2106del），另有兩個在LMNA基因中（NM_170707.3：c.73C>A和c.1209_1213dup）。

結論：在這十年病例回顧中，我們對心臟離子通道病和心肌病的香港華籍患者進行了基因分析。要正確解讀遺傳學結果需具備專業知識和經驗。在遺傳諮詢和直系家屬的連帶基因篩檢中，必須對非外顯性、變異表達性、表型-基因型相關性、易感性風險和二基因遺傳問題謹慎解讀。

TABLE 1. Common channelopathy- and cardiomyopathy-associated genes[2]

Disorder	Gene
Long and short QT syndrome	At least 12 genes: *KCNQ1, KCNH2, SCN5A, ANK2, AKAP9, CACNA1C, CAV3, KCNE1, KCNE2, KCNJ2, SCN4B,* and *SNTA1*
Brugada syndrome	At least 16 genes: *SCN5A, CACNA1C, CACNB2, CACNA2D1, GPD1L, HCN4, KCNE3, KCND3, KCNE1L (KCNE5), KCNJ8, RANGNRF, SCN1B, SCN2B, SCN3B, SLMAP,* and *TRPM4*
Catecholaminergic polymorphic ventricular tachycardia	2 Genes: *RYR2* and *CASQ2*
Hypertrophic cardiomyopathy	At least 24 genes: *ACTC1, ACTN2, CSRP3, FXN, GLA, JPH2, LAMP2, LDB3, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, PLN, PRKAG2, RAF1, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN,* and *VCL*
Dilated cardiomyopathy	At least 30 genes: *TTN4, LMNA, MYH7, MYH6, SCN5A, MYBPC3, TNNT2, BAG3, ANKRD1, RBM20, TMPO, LDB3, TCAP, VCL, TPM1, TNNI3, TNNC1, ACTC1, ACTN2, CSRP3, DES, NEXN, PSEN1, PSEN2, SGCD, EYA4, PLN, DSG2, DMD,* and *TAZ*
Arrhythmogenic right ventricular dysplasia/cardiomyopathy	At least 8 genes: *DSC2, DSG2, DSP, JUP, PKP2, RYR2, TGFB3,* and *TMEM43*

performed in the following order and stopped once a positive finding was detected: *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, and *SCN5A* for LQTS; *SCN5A* for Brugada syndrome; *RYR2* for CPVT; *MYH7* and *MYBPC3* for HCM; *LMNA* for DCM; and *PKP2* and *DSP* for ARVD/C. The order was based on prevalence according to the literature and local experience. All coding exons were amplified for each gene except selected exons 3, 8, 14, 45, 46, 47, 49, 88, 89, 90, 93, 96, 97, 100, 101, and 103 for *RYR2*.[6] The GenBank accession numbers are shown in Table 2. The pathogenicity of novel missense variants was analysed by Alamut Visual (Interactive Biosoftware, Rouen, France) with Polymorphism Phenotyping v2 (PolyPhen-2), Sorting Intolerant from Tolerant (SIFT), MutationTaster, and Assessing Pathogenicity Probability in Arrhythmia by Integrating Statistical Evidence (APPRAISE, https://cardiodb.org/APPRAISE/) and that of novel splicing variants by Splice Site Finder-like, MaxEntScan, NNSPLIC, GeneSplicer, and Human Splicing Finder, wherever appropriate. Splicing variants were considered to be damaging if there was a >10% lower score when compared with the wild-type prediction. Allele frequencies among populations were referred to the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/).

Results

During the 10-year study period more than 90 patients with channelopathies or cardiomyopathies were referred for genetic analysis. Among them, 28 unrelated patients had positive genetic results, comprising 17 males and 11 females. Their mean age at diagnosis was 39 years (range, 1-80 years). The major clinical presentations included syncope, palpitations, and abnormal electrocardiography (ECG) findings. Four patients were asymptomatic and were diagnosed following an incidental abnormal finding related to other medical issues. A family history was present in only 13 (46%) patients. All detected mutations were heterozygous, and 26 different heterozygous mutations were detected. These encompassed 11 missense, two nonsense, and five splicing mutations, as well as eight small insertions and deletions. There were six novel mutations—two in *SCN5A* (NM_198056.2:c.429del and c.2024-11T>A), two in *MYBPC3* (NM_000256.3:c.906-22G>A and c.2105_2106del), and two in *LMNA* (NM_170707.3:c.73C>A and c.1209_1213dup) [Table 3]. All were considered pathogenic or likely pathogenic according to the Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics by the Association for Clinical Genetic Science.[7] Further clinical details and genotypes are shown in Table 3.

There were seven patients with LQTS, two with Brugada syndrome, two with CPVT, nine with HCM, four with DCM, and four with ARVD/C. Three patients with LQTS had mutations in *KCNQ1* (cases 1-3) and four had mutations in *KCNH2* (cases 4-7). Two patients (cases 8 and 9) with Brugada syndrome had mutations in *SCN5A*, including two novel mutations. Two patients (cases 10 and 11) with CPVT had mutations in *RYR2*. Four patients with HCM (cases 12-15) had *MYH7* mutations and five (cases 16-20) had *MYBPC3* mutations, including two novel mutations. Four patients with DCM (cases 21-24) had *LMNA* mutations, including two novel mutations. Finally, three patients with ARVD/C had *PKP2* mutations (cases 25-27) and one had a *DSP* mutation (case 28).

Discussion

This is the first report of a cardiac genetic case series among Hong Kong Chinese patients with channelopathies and cardiomyopathies.

TABLE 2. Targeted genes in Sanger sequencing analysis

Gene	Encoding	GenBank accession numbers
KCNQ1	Potassium channel, voltage-gated, KQT-like subfamily, member 1	NG_008935.1, NM_000218.2, NP_000209.2
KCNH2	Potassium channel, voltage-gated, subfamily H, member 2	NG_008916.1, NM_000238.3, NP_000229.1
KCNE1	Potassium channel, voltage-gated, IsK-related family, member 1	NG_009091.1, NM_000219.4, NP_000210.2
KCNE2	Potassium channel, voltage-gated, IsK-related family, member 2	NG_008804.1, NM_172201.1, NP_751951.1
SCN5A	Sodium channel, voltage-gated, type V, alpha subunit	NG_008934.1, NM_198056.2, NP_932173.1
RYR2	Ryanodine receptor 2	NG_008799.1, NM_001035.2, NP_001026.2
MYH7	Myosin, heavy chain 7, cardiac muscle, beta (MIM *160760)	NG_007884.1, NM_000257.2, NP_000248.2
MYBPC3	Myosin-binding protein C, cardiac	NG_007667.1, NM_000256.3, NP_000247.2
LMNA	Lamin A/C	NG_008692.2, NM_170707.3, NP_733821.1
PKP2	Plakophilin 2	NG_009000.1, NM_004572.3, NP_004563.2
DSP	Desmoplakin (MIM *125647)	NG_008803.1, NM_004415.2, NP_004406.2

TABLE 3. Clinical and genetic findings of 28 Chinese patients with channelopathies and cardiomyopathies

Case No.	Sex/ age at diagnosis (years)	Clinical details	Family history	Gene	Heterozygous mutation detected	ExAC	PolyPhen-2 SIFT MutationTaster APPRAISE PrPP APPRAISE PoPP	HGMD accession	ClinVar
LQTS									
1.	M/8	Recurrent syncope induced by exercise and swimming. Long QTc 535 ms and broad-based T wave.	N	KCNQ1	c.1018T>C p.Phe340Leu	Absent	PD D DC 0.98 0.999	CM1511175	No entry
2.	M/9	Presented with an episode of unprovoked syncope. ECG showed long QTc at 490 ms. Started on beta-blocker and remained asymptomatic.	Y	KCNQ1	c.1032G>A p.Ala344=	Absent	Exon-skipping	CS992245	Pathogenic
3.	F/45	Asymptomatic. ECG showed long QTc at 505 ms. Followed up because of strong family history of sudden nocturnal death and prolonged QT interval.	Y	KCNQ1	c.1096C>T p.Arg366Trp	Absent	PD D DC 0.98 0.93	CM981130	Pathogenic/ likely pathogenic
4.	M/59	Symptomatic bradycardia with dizziness. One episode of loss of consciousness during sleep. Long QTc at 600 ms, with documented Torsades de pointes. ICD implanted	Y	KCNH2	c.211G>T p.Gly71Trp	Absent	PD D DC 0.96 0.995	CM1511185	No entry
5.	F/31	Asymptomatic, long QTc at 494 ms and on beta-blocker. One offspring with LQTS died of pneumonia in 2005.	Y	KCNH2	c.1750G>C p.Gly584Arg	Absent	PD D DC 0.96 >0.999	CM097452	Pathogenic
6.	M/1	Congenital muscular ventricular septal defect. Found bradycardia and long QTc at 560 ms during follow-up at 1 month old. Frequent runs of ventricular tachycardia, pulseless ventricular tachycardia with Torsades de pointes. Pacemaker implanted.	N	KCNH2	c.1883G>T p.Gly628Val	Absent	PD D DC 0.96 >0.999	CM055315	Pathogenic
7.	M/19	Found with severe nausea, flaccid limbs, eyes staring upwards, and frothiness in mouth in bed in the morning. Also had bilateral blurring of vision. Prolonged QTc at 550 ms. ICD implanted.	N	KCNH2	c.2892dup p.Gly965Argfs*154	Absent	Frameshift	CI097572	No entry
Brugada syndrome									
8.	M/26	Witnessed convulsion and found to have Brugada type I pattern on treadmill. ICD implanted.	N	SCN5A	c.429del † p.Asn144Thrfs*57	Absent	Frameshift	No entry	No entry
9.	F/61	Dizziness and syncope. ECG showed right bundle branch block and subtle ST elevation. Positive response to flecainide.	N	SCN5A	c.2024-11T>A †	Absent	Abolish the acceptor splicing site and create a cryptic site upstream	No entry	No entry
CPVT									
10.	F/10	Sudden cardiac arrest in school playground. Ventricular tachycardia and fibrillation in emergency department requiring multiple defibrillations and intensive care. Adrenaline infusion test revealed bidirectional premature ventricular beats and short runs of polymorphic ventricular tachycardia.	N	RYR2	c.11836G>A p.Gly3946Ser	Absent	PD D DC	CM024349	Pathogenic
11.	M/7	Exercise-induced recurrent loss of consciousness. Echocardiogram showed slightly thickened mitral valve and trivial mitral regurgitation. Isoprenaline infusion test suggested CPVT. On beta-blocker.	N	RYR2	c.14848G >A p.Glu4950Lys	Absent	B D DC	CM024354	No entry

Abbreviations: All = allele frequency in all populations; ARVD/C = arrhythmogenic right ventricular dysplasia/cardiomyopathy; B = benign predicted by PolyPhen-2; ClinVar = public archive of reports of the relationships among human variations and phenotypes (https://www.ncbi.nlm.nih.gov/clinvar/intro/); CPVT = catecholaminergic polymorphic ventricular tachycardia; D = damaging predicted by SIFT; DC = disease causing predicted by MutationTaster; DCM = dilated cardiomyopathy; EAS = allele frequency in East Asians; ECG = electrocardiography; ExAC = Exome Aggregation Consortium; HCM = hypertrophic cardiomyopathy; HGMD = Human Gene Mutation Database HGMD® Professional 2017.2; ICD = implantable cardioverter defibrillator; LQTS = long QT syndrome; MRI = magnetic resonance imaging; PD = probably damaging predicted by PolyPhen-2; PoD = possibly damaging predicted by PolyPhen-2; PolyPhen-2 = polymorphism phenotyping v2; PoPP = posterior probability of pathogenicity predicted by APPRAISE which is only available for LQTS; PrPP = prior probability of pathogenicity predicted by APPRAISE, only available for LQTS; SIFT = sorting intolerant from tolerant
† Novel mutation

TABLE 3. (cont'd)

Case No.	Sex/ age at diagnosis (years)	Clinical details	Family history	Gene	Heterozygous mutation detected	ExAC	PolyPhen-2 SIFT MutationTaster APPRAISE PrPP APPRAISE PoPP	HGMD accession	ClinVar
HCM									
12.	F/60	Detection of heart murmur during routine check-up. Incidental finding of obstructive HCM with mixed features of apical and classic HCM. One sibling and one offspring died of sudden cardiac death in their 30s. Her offspring had HCM.	Y	MYH7	c.1273G>A p.Gly425Arg	Absent	PD D DC	CM054791	Likely pathogenic/ uncertain significance
13.	M/57	Exertional chest pain. Found to have HCM. Refused to have prophylactic ICD implantation.	N	MYH7	c.1987C>T p.Arg663Cys	Absent	PD D DC	CM973126	Pathogenic/ likely pathogenic
14.	M/59	Irregular palpitations and hypertension. Echocardiogram showed HCM. One sibling had recurrent syncope and HCM with ICD implantation.	Y	MYH7	c.1987C>T p.Arg663Cys	Absent	PD D DC	CM973126	Pathogenic/ likely pathogenic
15.	F/48	Episode of syncope. Found to have HCM, with left ventricular dysfunction. Progressive deterioration in exercise tolerance. Heart transplantation done. One parent died of cardiomyopathy and sudden death in their 50s. One offspring with incidental finding of heart murmur and found to have HCM.	Y	MYH7	c.2155C>T p.Arg719Trp	Absent	PoD D DC	CM941086	Pathogenic
16.	M/52	Recurrent syncope, loss of consciousness, and episode of non-sustained ventricular tachycardia. Echocardiogram showed HCM. ICD implanted.	N	MYBPC3	c.906-22G>A †	All 0.00089% EAS 0%	Abolish the original acceptor splice site and create a cryptic site	No entry	No entry
17.	M/16	Sudden collapse and ventricular fibrillation, complicated by hypoxic brain damage. Echocardiogram showed HCM.	N	MYBPC3	c.1223+1G>A	Absent	Abolish the original donor splice site	No entry	Pathogenic
18.	F/43	History of palpitations. Incidental finding of pansystolic murmur at age 36 years. Echocardiogram showed asymmetrical intraventricular septal hypertrophy. Her parents died in their 40s of unknown cause.	N	MYBPC3	c.2105_2106del † p.Pro702Argfs*5	Absent	Frameshift	No entry	No entry
19.	F/20	Asymptomatic, incidental finding of heart murmur referred from student health service. Echocardiogram showed thickened left ventricular septum. One sibling also recently diagnosed with HCM but echocardiograms of both parents were normal.	Y	MYBPC3	c.2215G>A p.Glu739Lys	Absent	PD D DC	No entry	No entry
					c.3624del p.Lys1209Serfs*28	Absent	Frameshift	CD0910628	Pathogenic
20.	M/43	Atypical chest pain and epigastric discomfort. ECG showed typical features of HCM. Severe septal hypertrophy. One parent had HCM.	Y	MYBPC3	c.3624del p.Lys1209Serfs*28	Absent	Frameshift	CD0910628	Pathogenic
DCM									
21.	M/47	History of craniopharyngioma and found to have sick sinus syndrome and third-degree heart block on preoperative ECG. Echocardiography showed mildly dilated right ventricle, mild tricuspid regurgitation, mitral valve prolapse, and trivial mitral regurgitation. Several relatives had a history of atrioventricular block.	Y	LMNA	c.48_51dup p.Ser18Glnfs*24	Absent	Frameshift	CI117821	No entry
22.	F/50	Found unconscious at home. ECG showed ventricular fibrillation. Echocardiogram revealed DCM. Lower-limb weakness and muscle wasting in the past few years. One parent and one sibling died of chronic heart failure in their 30s or 40s. The deceased sibling also had heart block and muscular dystrophy.	Y	LMNA	c.73C>A † p.Arg25Ser	Absent	PD D DC	No entry	No entry
23.	M/50	Incidental finding of slow atrial flutter and heart block. Echocardiogram showed gross impairment of left ventricular function and heart MRI revealed anteroseptal and inferoseptal scars.	N	LMNA	c.1209_1213dup † p.His405Profs*77	Absent	Frameshift	No entry	No entry

TABLE 3. (cont'd)

Case No.	Sex/ age at diagnosis (years)	Clinical details	Family history	Gene	Heterozygous mutation detected	ExAC	PolyPhen-2 SIFT MutationTaster APPRAISE PrPP APPRAISE PoPP	HGMD accession	ClinVar
DCM (cont'd)									
24.	F/54	Progressive limb weakness and exertional dyspnoea. Mild elevation of plasma creatine kinase at around 1-2 times the normal upper limit. Echocardiogram showed dilated left atrium, mildly reduced left ventricular systolic function. Muscular dystrophy with cardiomyopathy and atrioventricular conduction disturbance. Put on pacemaker. Two siblings had similar conduction defects with permanent pacing and muscle weakness. Their muscle biopsies were unremarkable.	Y	LMNA	c.1609-1G>A	Absent	Abolish original acceptor splice site	CS1617015	Pathogenic/ likely pathogenic
ARVD/C									
25.	M/62	Sudden onset of fast palpitations. ECG at emergency department showed ventricular tachycardia. ICD implanted. Heart MRI showed right ventricular dysplasia. History of anterior acute myocardial infarction at age 48 years with percutaneous coronary angioplasty done.	N	PKP2	c.870G>A p.Trp290*	Absent	Nonsense	CM1312955	No entry
26.	M/80	Episodic palpitations with ECG showing paroxysmal ventricular tachycardia. Inducible ventricular tachycardia found on electrophysiological study. ICD implanted.	N	PKP2	c.1125_1132del p.Phe376Alafs*8	Absent	Frameshift	No entry	Pathogenic
27.	M/49	Recurrent palpitation attacks at age 39 years. Sudden onset of chest discomfort and palpitations associated with dizziness and found to have wide complex tachycardia. Cardiac catheterisation revealed focal aneurysm at right ventricular base. Electrophysiological study revealed inducible sustained ventricular tachycardia with two different morphologies. Heart MRI showed no focal or diffuse right ventricular wall thinning.	N	PKP2	c.1978C>T p.Gln660*	All 0.00083% EAS 0.012%	Nonsense	CM100599	No entry
28.	F/23	Good past health. Found collapsed in bathroom at home at night and death confirmed at emergency department arrival. Autopsy revealed mildly dilated ventricles and mottling of myocardium, with histological findings of circumferential fibrofatty infiltration of sub-epicardial region of left ventricle. Right ventricle was essentially normal.	N	DSP	c.2131_2132delAG p.Ser711Cysfs*4	Absent	Frameshift	CD1410462	Pathogenic/ likely pathogenic

A total of 28 patients are reported, and 26 different mutations and six novel mutations have been identified. Wide genetic diversity is observed, with no common mutation found. Hereditary channelopathies and cardiomyopathies are mainly inherited in an autosomal dominant manner. Mutations can be either inherited or de novo. Risk to proband sibling(s) and first-degree relatives depends on the genetic status of the parents. Offspring of the proband have a 50% risk of inheriting the mutation. Siblings of the proband have the same risk if the mutation is transmitted from either parent. Patients carrying a mutation of these sudden arrhythmia death syndromes show incomplete penetrance. In general, a mutation carrier will show symptoms/ signs in 80% of those with CPVT, 20% to 50% of ARVD/C patients, 18% to 63% of LQTS patients, 80% to 94% of SQTS patients, and 80% of patients with Brugada syndrome who have abnormal ECG findings when challenged with a sodium channel blocker.[8] No exact figure is available for HCM. The data could be more specific if a particular mutation was considered alongside clinical findings and family history. Pre-symptomatic testing of at-risk family members cannot be used to predict age of onset, severity, type of symptoms, or rate of progression. Detailed clinical, ECG, and genetic characterisation of affected and unaffected family members is helpful.

Long QT syndrome

Long QT syndrome is genetically heterogeneous, with at least 12 genes involved. Mutations in the four genes, *KCNQ1*, *KCNH2*, *KCNE1*, and *KCNE2*, are detected in 46%, 38%, 2%, and 1% of affected patients, respectively.[8] A small proportion of patients (3%) have double heterozygous mutations in more than one disease loci.[9] Specific arrhythmogenic triggers are associated with a particular subtype, such as exertion, swimming, and near-drowning for LQT1; auditory triggers and cardiac events occurring in the postpartum period for LQT2; and cardiac events during sleep or at rest for LQT3. Three patients had *KCNQ1* mutations. Case 1 had recurrent syncope induced by exercise and swimming, but genetic testing confirmed LQTS type 1. Other patients had no specific provoking factor. LQTS type 2 caused by *KCNH2* mutations accounts for about 38% of all LQTS.[8] Four patients (cases 4-7) carried *KCNH2* mutations and two (cases 4 and 6) presented with Torsades de pointes and one (case 7) had survived cardiac arrest requiring an implantable cardioverter defibrillator. Case 6 was the youngest patient, presenting at age 1 year. Genotype-guided treatment in LQTS is recommended and LQT1 responds best to beta-blockers.[10,11]

Brugada syndrome

Brugada syndrome is characterised by cardiac conduction abnormalities (ST-segment abnormalities in leads V1-V3 on ECG and a high risk for ventricular arrhythmias) that can result in sudden death. The Shanghai Score System has been recently published for the diagnosis of Brugada syndrome.[12,13] The prevalence of Brugada syndrome or its characteristic ECG pattern is reportedly higher among Asians, such as Japanese (0.14%-1.22%).[14-17]

Brugada syndrome is genetically heterogeneous and can be attributed to defects in at least 23 genes at the time of reporting.[8] Mutations in *SCN5A* are detected in 11% to 14% of affected individuals in Japan and <10% in Taiwan where mutations in *CACNA1C* account for 1% to 7%.[18] Approximately 65% to 70% of patients remain genetically undiagnosed. Expressivity is variable and penetrance is incomplete and low.

Conventionally, Brugada syndrome has been described as a monogenic disease that has autosomal dominant inheritance with incomplete penetrance; it is caused by rare genetic variants with a large effect size. Most individuals diagnosed with Brugada syndrome have an affected parent. The proportion of cases caused by a de-novo mutation is approximately 1%. Recent studies indicate that genetic inheritance is likely more complex, and models of an oligogenic disorder or susceptibility risk/genetic predisposition have been suggested.[19-22]

Among the two patients in this series, none had a positive family history. Symptoms were more non-specific, such as palpitation and syncope. It is noteworthy that convulsion can be a presentation of channelopathies (case 8). Clinical suspicion should be higher with more specific investigations, such as exercise-stress ECG and flecainide challenge tests, are required in order to reveal the real culprit. Sudden cardiac death can be the first presenting symptom in Brugada syndrome.

Two novel mutations are described in *SCN5A*: c.429del and c.2024-11T>A. The former is predicted to cause a frameshift and premature protein truncation. The latter is predicted to abolish the acceptor splice site and create a cryptic site upstream. At the time of reporting, both are absent from controls in the Exome Sequencing Project, 1000 Genomes Project, and ExAC. *SCN5A* mutations can cause either LQTS or Brugada syndrome.

Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia can present with syncope and sudden death during physical exertion or emotion, due to catecholamine-induced bidirectional ventricular tachycardia, polymorphic ventricular tachycardia or ventricular fibrillation. The reported mean age of onset is between 7 and 12 years.[8] Exercise stress testing or an adrenaline provocation test may induce ventricular arrhythmia and enable a clinical diagnosis. About half of these cases are related to a dominantly inherited *RYR2* gene mutation, with a small proportion (1%-2%) related to recessively inherited *CASQ2* gene mutations. *RYR2* is a large gene with 105 exons. Tier testing has been proposed by Medeiros-Domingo et al.[6] First-tier *RYR2* genetic testing of the 16 selected exons allows identification of about 65% of CPVT cases. There were two paediatric CPVT patients (cases 10 and 11) in our series, with two known disease-causing mutations detected, namely NM_001035.2(*RYR2*):c.11836G>A (p.Gly3946Ser)[23-26] and c.14848G>A (p.Glu4950Lys).[23,24] Both mutations were detected in first-tier screening.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is the most prevalent hereditary cardiac disease, causing about one third of sudden cardiac deaths in young athletes. Its prevalence in China is approximately 1 in 1250.[27] The clinical manifestations are markedly variable, ranging from asymptomatic to sudden cardiac death. Genetic testing provides an accurate diagnosis in the probands and enables screening of asymptomatic family members. Although the genetic background of HCM is heterogeneous, involving at least 30 genes, *MYH7* and *MYBPC3* are the most common and each accounts for approximately 40%.[8]

Nine patients with HCM are reported here: four had known *MYH7* mutations and five had *MYBPC3* mutations, including two novel mutations. NM_000256.3:c.906-22G>A was detected in case 16 and was a novel variant. Neither population frequency nor known pathogenicity have been reported. In-silico analysis showed creation of a novel acceptor site and insertion of 20 nucleotides into exon 10. This conceivably would lead to a frameshift and premature protein termination. Exon 10 of *MYBPC3* is a microexon in which the stability of its original splicing site is easily disrupted by intronic variants. A similar mutation has been reported as c.906-36G>A.[28] Nonetheless, cDNA analysis was not performed. NM_000256.3(*MYBPC3*):c.1223+1G>A at the critical canonical +1 splice site is also novel. In addition, other known disease-causing splicing mutations affecting the same nucleotide have been reported.[26,29,30] Case 19 had two variants detected in *MYBPC3* (c.2215G>A and c.3624del). The small deletion c.3624del is a mutation known to cause HCM in the Chinese population[31] and predicted to cause a frameshift and premature termination of the protein. The missense variant c.2215G>A is as yet unreported and is predicted by in-silico analyses to cause an amino acid change from glutamate to lysine at codon 739 and probably damage. At the time of reporting, the variant is absent from controls in the Exome Sequencing Project, 1000 Genomes Project, and ExAC databases. This variant is considered to have uncertain significance. The mother of the patient in case 19 was available for testing. She was 48 years old at the time of genetic testing, asymptomatic, and heterozygous for c.3624del only. Hence, the two variants c.2215G>A and c.3624del of *MYBPC3* were in-trans in the patient and elder brother of the patient in case 19. Both had a more severe form of HCM, with a younger onset.

Dilated cardiomyopathy

Familial DCM is a group of genetically heterogeneous disorders. Laminopathy can manifest as several allelic disorders affecting muscle, nerve, adipose, and vascular tissues; one of them is cardiomyopathy, dilated 1A. We identified four patients with DCM, two of whom also had proximal muscle weakness. Two novel mutations in *LMNA* were detected (c.73C>A and c.1209_1213dup). NM_005572.3(*LMNA*):c.73C>A is a novel variant that is predicted to be deleterious by SIFT, probably causing damage according to PolyPhen-2 and disease-causing according to MutationTaster. Other missense mutations have been reported in the same amino acid codon.[32-34] NM_005572.3(*LMNA*):c.1609-1G>A is predicted to significantly affect splicing by in-silico analysis. At the time of reporting, all variants are absent from controls in the Exome Sequencing Project, 1000 Genomes Project, and ExAC. In case

22 with NM_005572.3(*LMNA*):c.73C>A, one of the parents died of chronic heart failure in the fourth decade of life, and one sibling died of heart block and chronic heart failure with a diagnosis of muscular dystrophy at age 38 years. Nonetheless, there was no sample left for genotyping.

Arrhythmogenic right ventricular dysplasia/ cardiomyopathy

Arrhythmogenic right ventricular dysplasia/ cardiomyopathy is associated with fibrofatty replacement of cardiomyocytes, ventricular tachyarrhythmias, and sudden cardiac death. Although the right ventricle is primarily affected in this condition, left-dominant arrhythmogenic cardiomyopathy has also been described, and mutations have been identified in *DSP* as well as in other genes.[35] Four patients are reported here, with three having mutations in *PKP2* and one in *DSP*. Interestingly, the patient in case 26 presented at age 80 years with episodic palpitations. His ECG results showed paroxysmal ventricular tachycardia. He had a deletion in *PKP2*, c.1125_1132del (p.Phe376Alafs*8), resulting in a truncated incomplete protein product. Age of onset in patients with *PKP2* mutations is older than that of the patient with *DSP* mutation. The latter patient (case 28) died at age 23 years, with sudden collapse as the first presentation.

Primary arrhythmogenic disorders including LQTS/SQTS, CPVT, Brugada syndrome, and cardiomyopathies account for about one third of sudden cardiac deaths in the young.[36] Identification of a pathogenic variant can solve the diagnostic mystery, provide relief to the family, and enable family screening and counselling for other at-risk family members. In some developed countries, molecular autopsy is an essential part of a formal forensic investigation in unexplained sudden death.[37] We support the implementation of molecular autopsy in routine autopsy investigation of sudden cardiac death victims. Our group has conducted the first local prospective study to determine the prevalence and types of sudden arrhythmia death syndrome underlying sudden cardiac death among local young victims through clinical and molecular autopsy of sudden cardiac death victims and clinical and genetic evaluation of their first-degree relatives (http://www.sadshk.org/en/medical_research.php). Such data can serve as the groundwork for the feasibility of implementation of such investigations in Hong Kong.

Genetic tests for cardiac conditions can aid diagnosis and guide treatment. Nonetheless, there are limitations that complicate the translational use of genetic results in patient care, such as incomplete penetrance, variable expressivity, and findings of variants of uncertain significance. In addition, since the genetic heterogeneity is large among

cardiomyopathies and channelopathies and more genes are yet to be discovered, a negative genetic finding does not necessarily exclude a genetic basis of disease in patients.

Major limitations of the current study include its small sample size, incomplete family data for co-segregation study, and lack of functional study of novel variants. We observed a lower rate of use of genetic tests in early years that might have been due to insufficient awareness among clinicians about the clinical usefulness of such tests for channelopathies and cardiomyopathies. Clinical indications published in an expert consensus statement on the state of genetic testing for channelopathies and cardiomyopathies from the Heart Rhythm Society and European Heart Rhythm Association provide a good reference to determine when a genetic test should be requested.[5] In our hospital, referral information can be accessed on http://kwcpath. home/genetics/ and more information about genetic service provision in public hospitals is available in the Hong Kong Hospital Authority Genetic Test Formulary (http://gtf.home/). A comprehensive system of cardiac genetics service is required for an efficient referral system, resource funding, training, and appropriate long-term follow-up.

Conclusions

We present the phenotypic and genotypic characteristics of 28 unrelated Hong Kong Chinese patients diagnosed across a 10-year period. For each disease entity, it was beyond our reach in the past decade to exhaustively screen for all known genes. We therefore focus on the most common ones when investigating cardiac genetics. Even so, genetic analysis can provide an accurate diagnosis and is of utmost importance for the management of patients and their families. Non-penetrance, variable expressivity, phenotype-genotype correlation, susceptibility risk, and digenic inheritance have been reported. Genetic testing also allows for genetic counselling on the recurrence risk. Correct interpretation of genetic findings for careful genetic counselling requires professional expertise with relevant experience in both clinical medicine and molecular genetics. Next-generation sequencing will improve diagnostic performance in this genetically heterogeneous group of channelopathies and cardiomyopathies, and could become a mainstay diagnostic tool.

Author contributions

All authors have made substantial contributions to the concept or design of this study; acquisition of data; analysis or interpretation of data; drafting of the article; and critical revision for important intellectual content.

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